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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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DIAGNOSTIC AND THERAPEUTIC USE OF VAULT POLYNUCLEOTIDES AND PROTEINS FOR NEURODEGENERATIVE DISEASES

The present invention relates to methods of diagnosing, prognosticating and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social and economic burden. AD is the most common age-related neurodegenerative condition affecting about 10 % of the population over 65 years of age and up to 45 % over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently, this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people („aging of the baby boomers„) in developed countries. The neuropathological hallmarks that occur in the brains of individuals with AD are senile plaques, composed of amyloid-β protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles. AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the

progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-192).

Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon4 allele of apolipoprotein E (ApoE). Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP), presenilin-1, and presenilin-2, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents. It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to provide insight into the pathogenesis of neurodegenerative diseases and to provide methods, materials, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

Vaults are barrel-shaped ribonucleoprotein complexes of 13 Mega Dalton molecular weight. They are composed of three protein species and an untranslated RNA molecule called vault or vRNA. The three vault proteins are named major vault protein (or, alternatively, MVP, LRP, p100), minor vault protein TEP1 (p240), and minor vault protein ADPRTL1 (VPARP, PHP5, p193). The major vault protein is present in 96 copies per vault particle, and the minor vault proteins TEP1 and ADPRTL1 are found in 2 and 8 copies per particle, respectively (Kong et al., RNA

2000, 6: 890-900; Scheffer et al., Curr. Opin. Oncol. 2000, 12: 550-556). Vaults of nearly identical size and composition have been found in species as diverse as mammals, avians, amphibians, and the slime mold *Dictyostelium discoideum*. In spite of this evolutionary conserved and therefore apparently important function the exact cellular role of vaults is far from being understood. Several studies have implicated vaults in nucleocytoplasmic transport of different substrates including steroid hormone receptors and ribosomal particles (Scheffer et al., Curr. Opin. Oncol. 2000, 12: 550-556). Tissue distribution studies of vault components have shown higher levels of vaults in tissues that are chronically exposed to elevated levels of xenobiotics, in metabolically active tissue and in macrophages. An up-regulation of vault expression during the differentiation and maturation of human monocyte-derived dendritic cells, known as xenobiotic scavenging cells, has been observed in vitro (Scheffer et al., Curr. Opin. Oncol. 2000, 12: 550-556). Collectively, this argues for a prominent role of vaults in detoxification of tissues (Kickhoefer et al., J. Cell Biol. 1999, 146: 917-928; Scheffer et al., Curr. Opin. Oncol. 2000, 12: 550-556). Of outstanding medical relevance was the finding that vaults are overexpressed in multidrug-resistant cancer cell lines and expression levels of vault components correlated with the extent of drug resistance, again arguing for a role in transport of xenobiotics and tissue detoxification (Schoeijers et al., Cancer Res. 2000, 60: 1104-1110; Siva et al., Int. J. Cancer 2001, 92: 195-202; Scheffer et al., Curr. Opin. Oncol. 2000, 12: 550-556). In fact, the major vault protein has also been coined LRP, which stands for lung resistance-related protein (Scheper et al., Cancer Res. 1993, 53: 1475-1479).

While the major vault protein is thought to play a more structural role in particle assembly, the minor vault proteins seem to play more active, enzymatic roles. The minor vault protein TEP1 is shared with so called telomerase complexes, other ribonucleoprotein complexes essential for the maintenance of the length of the chromosomal telomeres in dividing cells (Kickhoefer et al.,

J. Biol. Chem. 1999, 274: 32712-32717; Collins, Curr. Opin. Cell Biol. 2000, 12: 378-383). However, thus far it has not been demonstrated that vaults have telomerase activity (Kickhoefer et al., J. Biol. Chem. 1999, 274: 32712-32717). The other minor vault protein, ADPRTL1, also called p193, offers a number of interesting homologies to well known cellular factors: an amino-terminal BRCT domain shared with proteins active in DNA repair, a poly (ADP-ribose) polymerase domain that may hold a function in cellular differentitiation, proliferation, tumor transformation, and recovery from DNA damage, an inter-alpha-trypsin inhibitor domain, a putative nuclear localization signal, and a carboxy-terminal domain for interaction with the major vault protein (Still et al., Genomics 1999, 62: 533-536; Kickhoefer et al., J. Cell Biol. 1999, 146: 917-928; Jean et al., FEBS Lett. 1999, 446: 6-8; Chiarugi, Trends Pharmacol. Sci. 2002, 23: 122-129). A patent application featuring purified human ADPRTL1/p193 nucleotide sequences, polypeptide sequences, and variants thereof, for the diagnosis and treatment of multidrug resistant cancer has been put forward (WO 99/62547). The nucleotide sequence of the gene coding for ADPRTL1 was initially determined by Still, et al. (Genomics 1999, 62:533-536) and deposited in the GenBank database with the accession number AF057160. The ADPRTL1 gene codes for a polypeptide of 1724 amino acids in length with a predicted molecular weight of 193 kDa.

It is primarily the combination of the BRCT domain with the poly(ADP-ribose) polymerase domain that fuels interest in ADPRTL1. It was found that ADPRTL1 poly(ADP-ribosyl)ates itself and the major vault protein at the expense of nicotineamide adenine dinucleotide (Kickhoefer et al., J. Cell Biol. 1999, 146: 917-928). This activity resembles the enzymatic activity of a large family of poly(ADP-ribose) polymerases or PARPs (Johansson, Genomics 1999, 57: 442-445; Chiarugi, Trends Pharmacol. Sci. 2002, 23: 122-129). PARPs sense DNA damage and participate in DNA excision repair. Upon binding to DNA strand breaks, PARPs polymerize nicotineamide adenine dinucleotides into branched polymers of ADP-

ribose that are transferred to nuclear housekeeping proteins including DNA polymerase I and II, Ca^{2+}-Mg^{2+}-endonuclease, histones, chromatin-binding proteins, and the PARPs themselves (for review, Chiarugi, Trends Pharmacol. Sci. 2002, 23: 122-129). These modifications are thought to facilitate the repair process of the DNA. Excessive activation of PARPs may ultimately drive cells into energy crisis due to depletion of nicotineamide adenine dinucleotide pools and eventually elicit cell death. In fact, small-molecule inhibitors of PARPs hold therapeutic promise as anti-apoptotic drugs (Szabo et al., Trends Pharmacol. Sci. 1998, 19: 287-298; Pieper et al., Trends Pharmacol. Sci. 1999, 20: 171-181).

Kickhoefer and coworkers analysed the distribution of ADPRTL1/p193 messenger RNA in human tissues and found a prominent transcript in kidney, spleen, and liver but no transcript in brain (Kickhoefer et al., J. Cell Biol. 1999, 146: 917-928). In the present invention, using an unbiased and sensitive differential display approach, an ADPRTL1 transcript is detected in human brain samples. Importantly, the present invention discloses an up-regulation of ADPRTL1 transcripts in the inferior temporal lobe of brain samples taken from AD patients relative to frontal cortex samples. No such up-regulation is observed in samples from age-matched healthy controls. To date, no experiments have been described that show a relationship between a differential expression of the ADPRTL1 gene and the pathology of neurodegenerative diseases, particularly AD. Likewise, no experiments have been described that demonstrate a link between the dysregulation of vault gene expression and neurodegenerative disorders. Such a link offers new ways, inter alia, for the diagnosis and treatment of said disorders, in particular AD.

The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example, "a cell" means as well a plurality of cells, and so forth. The term „and/or" as used in the present specification and in the claims implies

that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording „determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term „level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term „activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of biologically active molecules. The term „activity" also refers to enzymatic activity. The terms „level" and/or „activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. A gene product comprises either RNA or protein and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term „gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). The term „fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term „derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a „derivative" may be generated by processes such as altered phosphorylation, or glycosylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term „modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the

activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a „modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene. The term 'AD' shall mean Alzheimer's disease.

Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, cerebrovascular dementia, multiple system atrophy, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, ischemic stroke, age-related macular degeneration and narcolepsy.

In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is compared to a reference value representing a known disease or health status. Thereby the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

In a preferred embodiment, said subjects suffer from Alzheimer's disease.

It is preferred that said vault protein is a minor vault protein, particularly minor vault protein ADPRTL1, also called p193. The present invention discloses the differential expression and regulation of the minor vault protein ADPRTL1 gene in specific brain regions of AD patients. Consequently, the minor vault protein ADPRTL1 gene and its corresponding translation products may have a causative role in the regional selective neuronal degeneration typically observed in AD. Alternatively,

the minor vault protein ADPRTL1 may confer a neuroprotective function to the remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular AD. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

It is particularly preferred that said sample to be analyzed and determined is selected from the group consisting of a brain tissue or other tissues, organs or body cells. The sample can also consist of cerebrospinal fluid or other body fluids including saliva, urine, blood, serum plasma, or nasal mucosa.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

In preferred embodiments, an increase or decrease in ADPRTL1 mRNA and /or ADPRTL1 protein in a sample cell or tissue from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of becoming diseased with a neurodegenerative disease, particularly AD.

In preferred embodiments, measurement of the level of transcription products of a gene coding for a vault protein is performed in a sample from a subject using a quantitative PCR-analysis with primer combinations to amplify said gene specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. A

Northern blot with probes specific for said gene can also be applied. It might further be preferred to measure transcription products by means of chip-based micro-array technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000).

Furthermore, a level and/or activity of a translation product of a gene coding for a vault protein and/or fragment of said translation product can be detected using an immunoassay, an activity assay, and/or binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999). All these detection techniques may also be employed in the format of micro-arrays, protein-arrays, or protein-chip based technologies.

In a preferred embodiment, the level, or the activity, or both said level and said activity of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment or derivative of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. In yet another

preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating neurodegenerative diseases, in particular AD, in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, in particular AD, said kit comprising:
(a) at least one reagent which is selected from the group consisting of (I) reagents that selectively detect a transcription product of a gene coding for a vault protein (II) reagents that selectively detect a translation product of a gene coding for a vault protein; and
(b) instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of a subject to develop such a disease by
- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of a gene coding for a vault protein, in a sample from said subject; and
- diagnosing or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of said subject to develop such a disease,
wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of a neurodegenerative disease, in particular AD, or an increased propensity or predisposition of developing such a disease. The kit, according to the present invention, may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerative disease, in particular AD. Consequently, the kit, according to the invention, may serve as a means for targeting identified individuals for early

preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of a neurodegenerative disease, in particular AD in a subject, as well as monitoring success or failure of therapeutic treatment for such a disease of said subject.

In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular AD, in a subject comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein, and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment or derivative of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence of a translation product of a gene coding for a vault protein, or a fragment, or derivative, or a variant thereof.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant

viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane pertubation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514; the contents of which are incorporated herein by reference). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against transcripts of a gene coding for a vault protein, particularly the minor vault protein ADPRTL1. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligo-deoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs

the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy.

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection, liposomal mediated transfection.

In preferred embodiments, said agent for treating and preventing a neurodegenerative disease, in particular AD, is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or da-

maged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of the adult brain are free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neurodegenerative disorder, e.g. a transgenic mouse and/or a knock-out mouse with an AD-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment or derivative of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein, and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment or derivative of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment or derivative of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular AD.

In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native gene sequence coding for a vault protein, or a fragment thereof, or a derivative thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of

a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of neuropathology similar to a neurodegenerative disease, in particular AD. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., 1994, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular AD. In preferred embodiments, said recombinant, non-human animal comprises a non-native gene sequence coding for a vault protein, in particular the non-native minor vault protein ADPRTL1 gene sequence, or a fragment thereof.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein, and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment or derivative of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the level, or both the activity and the level of one or more sub-

stances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein, and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment or derivative of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level of said substances in a matched control animal which is equally predisposed to developing or has already developed said diseases and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses a gene coding for a vault protein, or a fragment thereof, or a derivative thereof, under the control of a transcriptional regulatory element which is not the native vault protein gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and a vault protein, or a fragment or derivative thereof. Said screening assay comprises the steps of (i) adding a liquid suspension of said vault protein, or a fragment or derivative thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding fluorescently labelled ligand to said containers, and (iv) incubating said vault protein, or said fragment or derivative thereof, and said compound or plurality of compounds, and said fluorescently labelled ligand, and (v) measuring the amounts of fluorescence associated with said vault protein, or with said fragment or derivative thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said vault protein, or said fragment or derivative thereof. Instead of utilizing a fluorescently labelled ligand, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive labels, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to a gene product of a gene coding for a vault protein, or a fragment or derivative thereof. In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a gene product of a gene coding

for a vault protein by the aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to a vault protein, or to a fragment or derivative thereof. Said screening assay comprises (i) adding a liquid suspension of said vault protein, or a fragment or derivative thereof, to a plurality of containers, and (ii) adding a fluorescently labelled compound or a plurality of fluorescently labelled compounds to be screened for said binding to said plurality of containers, and (iii) incubating said vault protein, or said fragment or derivative thereof, and said fluorescently labelled compound or fluorescently labelled compounds, and (iv) measuring the amounts of fluorescence associated with said vault protein, or with said fragment or derivative thereof, and (v) determining the degree of binding by one or more of said compounds to said vault protein, or said fragment or derivative thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to a vault protein.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a gene product of a gene coding for a vault protein by the aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

In all types of assays disclosed herein it is preferred to study a vault protein. It is particularly preferred to conduct screening assays with the minor vault protein ADPRTL1.

The present invention features an antibody which is specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of a gene coding for a vault protein, in particular the minor vault protein ADPRTL1, or a fragment thereof. The immunogen may comprise immunogenic or antigenic epitopes or portions of a translation product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The term „antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized, or single chain antibodies, as well as fragments thereof. Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods involving detecting translation products of a gene coding for a vault protein, in particular the minor vault protein ADPRTL1.

In a preferred embodiment of the present invention, said antibodies can be used for detecting the pathological state of a cell in a sample from a

subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. Preferably, the pathological state relates to a neurodegenerative disease, in particular to AD. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the method described in US patent 6150173).

Other features and advantages of the invention will be apparent form the following description of figures and examples which are illustrative only and not intended to limit the remainder of the disclosure in any way.

Figure 1 depicts the brain regions with selective vulnerability to neuronal loss and degeneration in AD. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in AD (Terry et al., *Annals of Neurology* 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in AD. Brain tissues from the frontal cortex (F) and the temporal cortex (T) of AD patients and healthy, age-matched control individuals were used for the herein disclosed examples. For illustrative purposes, the image of a normal healthy brain was taken from a publication by

Strange (*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p.4).

Figure 2 discloses the initial identification of the differential expression of the human gene coding for minor vault protein ADPRTL1 in a fluorescence differential display screen. The figure shows a clipping of a large preparative fluorescent differential display gel. PCR products from the frontal cortex (F) and the temporal cortex (T) of two healthy control subjects and six AD patients were loaded in duplicate onto a denaturing polyacrylamide gel (from left to right). PCR products were obtained by amplification of the individual cDNAs with the corresponding one-base-anchor oligonucleotide and the specific Cy3 labelled random primers. The arrow indicates the migration position where significant differences in intensity of the signals for human minor vault protein ADPRTL1 transcript derived from frontal cortex as compared to the signals derived from the temporal cortex of AD patients exist. The differential expression reflects an up-regulation of human minor vault protein ADPRTL1 gene transcription in the temporal cortex compared to the frontal cortex of AD patients. Comparing the signals derived from temporal cortex and frontal cortex of healthy non-AD control subjects with each other, no difference in signal intensity, i.e. no altered expression level can be detected.

Figure 3 illustrates the verification of the differential expression of the minor vault protein ADPRTL1 gene by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and temporal cortex (T) of AD patients (Fig 3a) and of healthy, age-matched control individuals (Fig 3b) was performed by the LightCycler rapid thermal cycling technique. The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for the ribosomal protein S9,

the transferrin receptor, GAPDH, and beta-actin. The figure depicts the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of the minor vault protein ADPRTL1 cDNA from both the frontal and temporal cortices of a normal control individual during the exponential phase of the reaction overlap (Fig 3b, arrow), whereas in AD (Fig 3a, arrows), there is a significant shift of the curve for the sample derived from temporal cortex, indicating an up-regulation of the minor vault protein ADPRTL1 mRNA expression in temporal cortex relative to frontal cortex.

Figure 4 depicts SEQ ID NO: 1, the nucleotide sequence of the 35 bp minor vault protein ADPRTL1 cDNA fragment, identified and obtained by fluorescence differential display and subsequent cloning.

Figure 5 charts the schematic alignment of SEQ ID NO: 1 to the nucleotide sequence of the minor vault protein ADPRTL1 cDNA (GenBank accession number AF057160). The open rectangle represents the minor vault protein ADPRTL1 open reading frame, thin bars represent the 5' and 3' untranslated regions (UTRs).

Figure 6 outlines the sequence alignment of SEQ ID NO: 1 to the nucleotide sequence of the minor vault protein ADPRTL1 cDNA (GenBank accession number AF057160).

Table 1 lists the gene expression levels in the temporal cortex relative to the frontal cortex for the minor vault protein ADPRTL1 gene in seven AD patients (0.91 to 1.69 fold) and five healthy, age-matched control individuals (0.77 to 1.23 fold).

EXAMPLE I:

(i) Brain tissue dissection from patients with AD:

Brain tissues from AD patients and age-matched control subjects were collected within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see Fig. 1) and stored at -80 °C until RNA extractions were performed.

(ii) Isolation of total mRNA:

Total RNA was extracted from post-mortem brain tissue by using the RNeasy kit (Qiagen) according to the manufacturer's protocol. The accurate RNA concentration and the RNA quality were determined with the DNA LabChip system using the Agilent 2100 Bioanalyzer (Agilent Technologies). For additional quality testing of the prepared RNA, i.e. exclusion of partial degradation and testing for DNA contamination, specifically designed intronic GAPDH oligonucleotides and genomic DNA as reference control were utilised to generate a melting curve with the LightCycler technology as described in the supplied protocol by the manufacturer (Roche).

(iii) cDNA synthesis and identification of differentially expressed genes by fluorescence differential display (FDD):

In order to identify changes in gene expression in different tissues we employed a modified and improved differential display (DD) screening method. The original DD screening method is known to those skilled in the art (Liang and Pardee, *Science* 1995, 267:1186-7). This technique compares two populations of RNA and provides clones of genes that are expressed in one population but not in the other. Several samples can be analyzed simultaneously and both up- and down-regulated genes can

be identified in the same experiment. By adjusting and refining several steps in the DD method as well as modifying technical parameters, e.g. increasing redundancy, evaluating optimized reagents and conditions for reverse transcription of total RNA, optimizing polymerase chain reactions (PCR) and separation of the products thereof, a technique was developed which allows for highly reproducible and sensitive results. The applied and improved DD technique was described in detail by von der Kammer et al. (*Nucleic Acids Research* 1999, 27: 2211-2218). A set of 64 specifically designed random primers were developed (standard set) to achieve a statistically comprehensive analysis of all possible RNA species. Further, the method was modified to generate a preparative DD slab-gel technique, based on the use of fluorescently labelled primers. In the present invention, RNA populations from carefully selected post-mortem brain tissues (frontal and temporal cortex) of AD patients and age-matched control subjects were compared.

As starting material for the DD analysis we used total RNA, extracted as described above (ii). Equal amounts of 0.05 µg RNA each were transcribed into cDNA in 20 µl reactions containing 0.5 mM each dNTP, 1 µl Sensiscript Reverse Transcriptase and 1x RT buffer (Qiagen), 10 U RNase inhibitor (Qiagen) and 1 µM of either one-base-anchor oligonucleotides $HT_{11}A$, $HT_{11}G$ or $HT_{11}C$ (Liang et al., *Nucleic Acids Research* 1994, 22: 5763-5764; Zhao et al., *Biotechniques* 1995, **18:** 842-850). Reverse transcription was performed for 60 min at 37 °C with a final denaturation step at 93 °C for 5 min. 2 µl of the obtained cDNA each was subjected to a polymerase chain reaction (PCR) employing the corresponding one-base-anchor oligonucleotide (1 µM) along with either one of the Cy3 labelled random DD primers (1 µM), 1x GeneAmp PCR buffer (Applied Biosystems), 1.5 mM $MgCl_2$ (Applied Biosystems), 2 µM dNTP-Mix (dATP, dGTP, dCTP, dTTP Amersham Pharmacia Biotech), 5 % DMSO (Sigma), 1 U AmpliTaq DNA Polymerase (Applied Biosystems) in a 20 µl final volume. PCR conditions were set as follows: one round at 94 °C for

30 sec for denaturing, cooling 1 °C/sec down to 40 °C, 40 °C for 4 min for low-stringency annealing of primer, heating 1 °C/sec up to 72 °C, 72 °C for 1 min for extension. This round was followed by 39 high-stringency cycles: 94 °C for 30 sec, cooling 1 °C/sec down to 60 °C, 60 °C for 2 min, heating 1 °C/sec up to 72 °C, 72 °C for 1 min. One final step at 72 °C for 5 min was added to the last cycle (PCR cycler: Multi Cycler PTC 200, MJ Research). 8 µl DNA loading buffer were added to the 20 µl PCR product preparation, denatured for 5 min and kept on ice until loading onto a gel. 3.5 µl each were separated on 0.4 mm thick, 6 %-polyacrylamide (Long Ranger)/ 7 M urea sequencing gels in a slab-gel system (Hitachi Genetic Systems) at 2000 V, 60W, 30 mA, for 1 h 40 min. Following completion of the electrophoresis, gels were scanned with a FMBIO II fluorescence-scanner (Hitachi Genetic Systems), using the appropriate FMBIO II Analysis 8.0 software. A full-scale picture was printed, differentially expressed bands marked, excised from the gel, transferred into 1.5 ml containers, overlayed with 200 µl sterile water and kept at –20°C until extraction.

Elution and reamplification of DD products: The differential bands were extracted from the gel by boiling in 200 µl H_2O for 10 min, cooling down on ice and precipitation from the supernatant fluids by using ethanol (Merck) and glycogen/sodium acetate (Merck) at – 20 °C over night, and subsequent centrifugation at 13.000 rpm for 25 min at 4 °C. Pellets were washed twice in ice-cold ethanol (80%), resuspended in 10 mM Tris pH 8.3 (Merck) and dialysed against 10 % glycerol (Merck) for 1 h at room temperature on a 0.025 µm VSWP membrane (Millipore). The obtained preparations were used as templates for reamplification by 15 high-stringency cycles in 25-µl PCR mixtures containing the corresponding primer pairs as used for the DD PCR (see above) under identical conditions, with the exception of the initial round at 94 °C for 5 min, followed by 15 cycles of: 94 °C for 45 sec, 60 °C for 45 sec, ramp 1°C/sec to 70 °C for 45 sec, and one final step at 72 °C for 5 min.

Cloning and sequencing of DD products: Re-amplified cDNAs were analyzed with the DNA LabChip® system (Agilent 2100 Bioanalyzer, Agilent Technologies) and ligated into the pCR-Blunt II-TOPO vector and transformed into *E.coli* Top10F' cells (Zero Blunt TOPO PCR Cloning Kit, Invitrogen) according to the manufacturer's instructions. Cloned cDNA fragments were sequenced by commercially available sequencing facilities. The result of one such FDD experiment for the human minor vault protein ADPRTL1 gene is shown in Fig. 2.

(iv) Confirmation of differential expression by quantitative RT-PCR:

Positive corroboration of differential expression of the human minor vault protein ADPRTL1 gene was performed using the LightCycler technology (Roche). This technique features rapid thermal cyling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and therefore allows for highly accurate quantification of RT-PCR products by using a kinetic, rather than an endpoint readout. The ratio of human minor vault protein ADPRTL1 cDNA from the temporal cortex and frontal cortex was determined (relative quantification).

First, a standard curve was generated to determine the efficiency of the PCR with specific primers for the human minor vault protein ADPRTL1 gene (5'-GATGCTGTGCCTTGGACAGAA-3' and 5'-TGGTGTAAGTTTCCAGAAGCCA-3'). PCR amplification (95 °C and 1 sec, 56 °C and 5 sec, and 72 °C and 5 sec) was performed in a volume of 20 µl containing LightCycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM $MgCl_2$; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and, depending on the primers used, additional 3 mM $MgCl_2$. Melting curve analysis revealed a single peak at approximately 82°C with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system

(Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 66 bp for the minor vault protein ADPRTL1 gene was observed in the electropherogram of the sample.

In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTTTGCC-3' except for $MgCl_2$ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'- TCTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM $MgCl_2$ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' and 5'-GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGGCTGTTGTACCTCTC-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

For calculation of the values, first the logarithm of the cDNA concentration was plotted against the threshold cycle number C_t for minor vault protein ADPRTL1 and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNAs from frontal cortex and temporal cortex were analyzed in parallel and normalized to cyclophilin B. The C_t values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10 \wedge ((C_t \text{ value} - \text{intercept}) / \text{slope}) \quad [\text{ng total brain cDNA}]$$

The values for frontal and temporal cortex cDNAs of minor vault protein ADPRTL1 cDNA were normalized to cyclophilin B, and the ratio was calculated using the following formula:

$$\text{Ratio} = \frac{\text{ADPRTL1 temporal [ng]} / \text{cyclophilin B temporal [ng]}}{\text{ADPRTL1 frontal [ng]} / \text{cyclophilin B frontal [ng]}}$$

In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the temporal to frontal ratios of expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in step 2 and step 3, and the ratio from one gene to another gene remained constant in different runs, it was possible to normalize the values for the minor vault protein ADPRTL1 to the mean average value of the set of reference standard genes instead of normalizing to one single gene alone. The calculation was performed by dividing the ratio shown above by the deviation of cyclophilin B from the mean value of all housekeeping genes. The results of one such quantitative RT-PCR analysis for the minor vault protein ADPRTL1 gene are shown in Figure 3.

CLAIMS

1. A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising:

determining a level and/or an activity of

(i) a transcription product of a gene coding for a vault protein, and/or

(ii) a translation product of a gene coding for a vault protein and/or

(iii) a fragment or derivative of said transcription or translation product

in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

2. A method of monitoring the progression of a neurodegenerative disease in a subject, comprising:

determining a level and/or an activity of

(i) a transcription product of a gene coding for a vault protein, and/or

(ii) a translation product of a gene coding for a vault protein, and/or

(iii) a fragment or derivative of said transcription or translation product

in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby monitoring the progression of said neurodegenerative disease in said subject.

3. A method of evaluating a treatment for a neurodegenerative disease, comprising:

determining a level and/or an activity of

(i) a transcription product of a gene coding for a vault protein, and/or

(ii) a translation product of a gene coding for a vault protein, and/or

(iii) a fragment or derivative of said transcription or translation product

in a sample from a subject being treated for said disease and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby evaluating said treatment for said neurodegenerative disease.

4. The method according to any of claims 1 to 3 wherein said neurodegenerative disease is Alzheimer's disease.

5. The method according to any of claims 1 to 4 wherein said vault protein is a minor vault protein, in particular the minor vault protein ADPRTL1.

6. The method according to any of claims 1 to 5 wherein said sample is a cell, or a tissue, or an organ, or a body fluid, in particular cerebrospinal fluid or blood.

7. The method according to any of claims 1 to 6 wherein said reference value is that of a level and/or an activity of

(i) a transcription product of a gene coding for a vault protein, and/or

(ii) a translation product of a gene coding for a vault protein, and/or

(iii) a fragment or derivative of said transcription or translation product

in a sample from a subject not suffering from said neurodegenerative disease.

8. The method according to any of claims 1 to 7 wherein an increase or decrease in a transcription product of the gene coding for the minor

vault protein ADPRTL1 and/or a translation product of a gene coding for the minor vault protein ADPRTL1 in a cell, or tissue, or body fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

9. The method according to any of claims 1 to 8, further comprising comparing a level and/or an activity of

(i) a transcription product of a gene coding for a vault protein, and/or

(ii) a translation product of a gene coding for a vault protein, and/or

(iii) a fragment or derivative of said transcription or translation product

in a series of samples taken from said subject over a period of time.

10. The method according to claim 9 wherein said subject receives a treatment prior to one or more of said sample gatherings.

11. The method according to claim 10 wherein said level and/or activity is determined before and after said treatment of said subject.

12. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of a gene coding for a vault protein and (ii) reagents that selectively detect a translation product of a gene coding for a vault protein and

(b) an instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of a subject to develop such a disease by (i) detecting a level, or an activity, or both said level and said

activity, of said transcription product and/or said translation product of a gene coding for a vault protein, in a sample from said subject; and (ii) diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of said subject to develop such a disease, wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status indicates a diagnosis or prognosis of a neurodegenerative disease, in particular Alzheimer's disease, or an increased propensity or predisposition of developing such a disease.

13. A method of treating or preventing a neurodegenerative disease, in particular AD, in a subject comprising administering to said subject in a therapeutically or prophylactically effective amount an agent or agents which directly or indirectly affect an activity and/or a level of

(i) a gene coding for a vault protein, and/or
(ii) a transcription product of a gene coding for a vault protein, and/or
(iii) a translation product of a gene coding for a vault protein, and/or
(iv) a fragment or derivative of (i) to (iii).

14. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of

(i) a gene coding for a vault protein and/or
(ii) a transcription product of a gene coding for a vault protein and/or
(iii) a translation product of a gene coding for a vault protein, and/or
(iv) a fragment or derivative of (i) to (iii).

15. A recombinant, non-human animal comprising a non-native gene sequence coding for a vault protein or a fragment thereof, or a derivative thereof, said animal being obtainable by:

(i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and

(ii) introducing said targeting construct into a stem cell of a non-human animal, and

(iii) introducing said non-human animal stem cell into a non-human embryo, and

(iv) transplanting said embryo into a pseudopregnant non-human animal, and

(v) allowing said embryo to develop to term, and

(vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and

(vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing a neurodegenerative disease or related diseases or disorders.

16. The animal according to claim 15 wherein said vault protein is a minor vault protein, in particular the minor vault protein ADPRTL1.

17. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) a gene coding for a vault protein, and/or

(ii) a transcription product of a gene coding for a vault protein, and/or

(iii) a translation product of a gene coding for a vault protein, and/or

(iv) a fragment or derivative of (i) to (iii),

said method comprising:

(a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

(d) comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

18. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) a gene coding for a vault protein, and/or

(ii) a transcription product of a gene coding for a vault protein, and/or

(iii) a translation product of a gene coding for a vault protein, and/or

(v) a fragment or derivative of (i) to (iii),

said method comprising:

(a) administering a test compound to a test animal which is predisposed to developing or has already developed a neurodegenerative disease or related diseases or disorders in respect of the substances recited in (i) to (iv);

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) or (iv) in a matched control animal which is predisposed to developing or has already developed a neurodegenerative disease or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which animal no such test compound has been administered;

(d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases or disorders.

19. The method according to claim 18 wherein said test animal and/or said control animal is a recombinant animal which expresses a vault protein, or a fragment thereof, or a derivative thereof, under the control of a transcriptional control element which is not the native vault protein gene transcriptional control element.

20. An assay for testing a compound, preferably for screening a plurality of compounds for inhibition of binding between a ligand and a vault protein, or a fragment or derivative thereof, said assay comprising the steps of:

(i) adding a liquid suspension of said vault protein, or a fragment or derivative thereof, to a plurality of containers;

(ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers;

(iii) adding fluorescently labelled ligand to said containers;

(iv) incubating said vault protein, or said fragment or derivative thereof, and said compound or compounds, and said fluorescently labelled ligand;

(v) measuring amounts of fluorescence associated with said vault protein, or with said fragment or derivative thereof; and

(vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said vault protein, or said fragment or derivative thereof.

21. An assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to

a vault protein, or to a fragment or derivative thereof, said assay comprising the steps of:

(i) adding a liquid suspension of said vault protein, or a fragment or derivative thereof, to a plurality of containers;

(ii) adding a fluorescently labelled compound or a plurality of fluorescently labelled compounds to be screened for said binding to said plurality of containers;

(iii) incubating said vault protein, or said fragment or derivative thereof, and said fluorescently labelled compound or fluorescently labelled compounds;

(iv) measuring amounts of fluorescence associated with said vault protein, or with said fragment or derivative thereof; and

(v) determining the degree of binding by one or more of said compounds to said vault protein, or said fragment or derivative thereof.

22. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of a gene coding for a vault protein, in particular the minor vault protein ADPRTL1, or a fragment thereof, for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell, and wherein said pathological state relates to a neurodegenerative disease, in particular Alzheimer's disease.

ABSTRACT

The present invention discloses the differential expression of the minor vault protein ADPRTL1 gene in specific brain regions of Alzheimer's disease patients. Based on this finding, this invention provides a method for diagnosing or prognosticating Alzheimer's disease in a subject, or for determining whether a subject is at increased risk of developing Alzheimer's disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using a gene coding for a vault protein, in particular the gene coding for the minor vault protein ADPRTL1. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.

Figure 1: Identification of genes involved in Alzheimer's Disease pathology

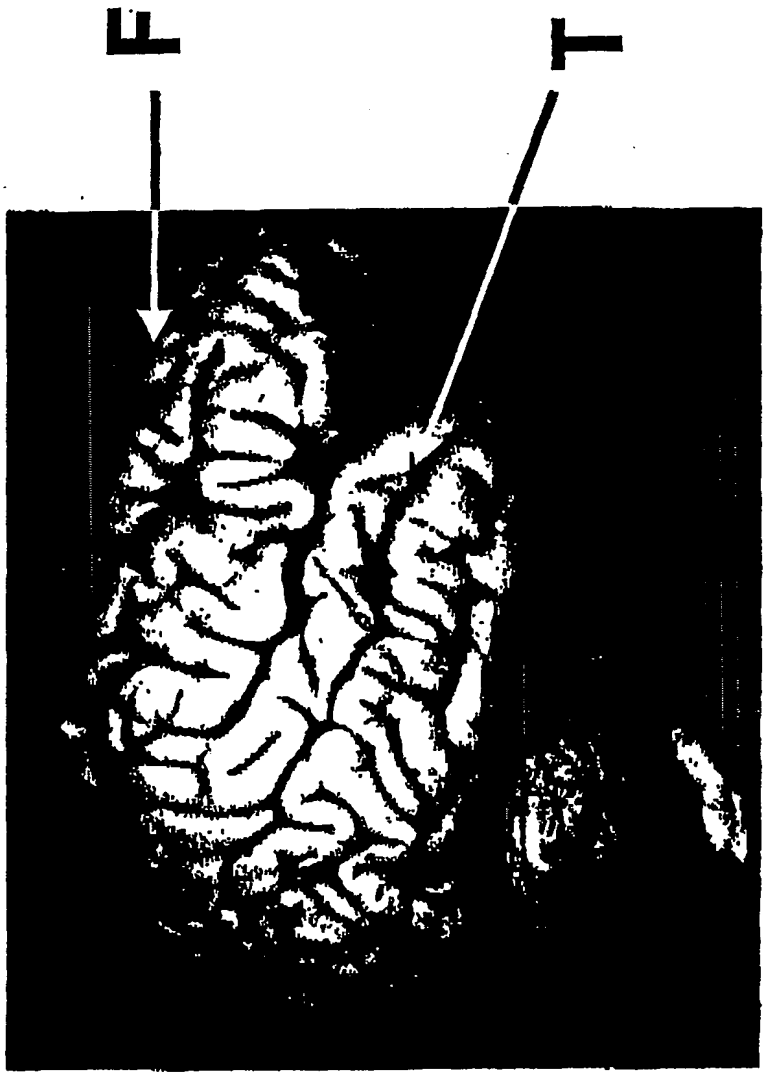

Figure 2: Identification of differentially expressed Alzheimer's disease genes in a fluorescence differential display screen

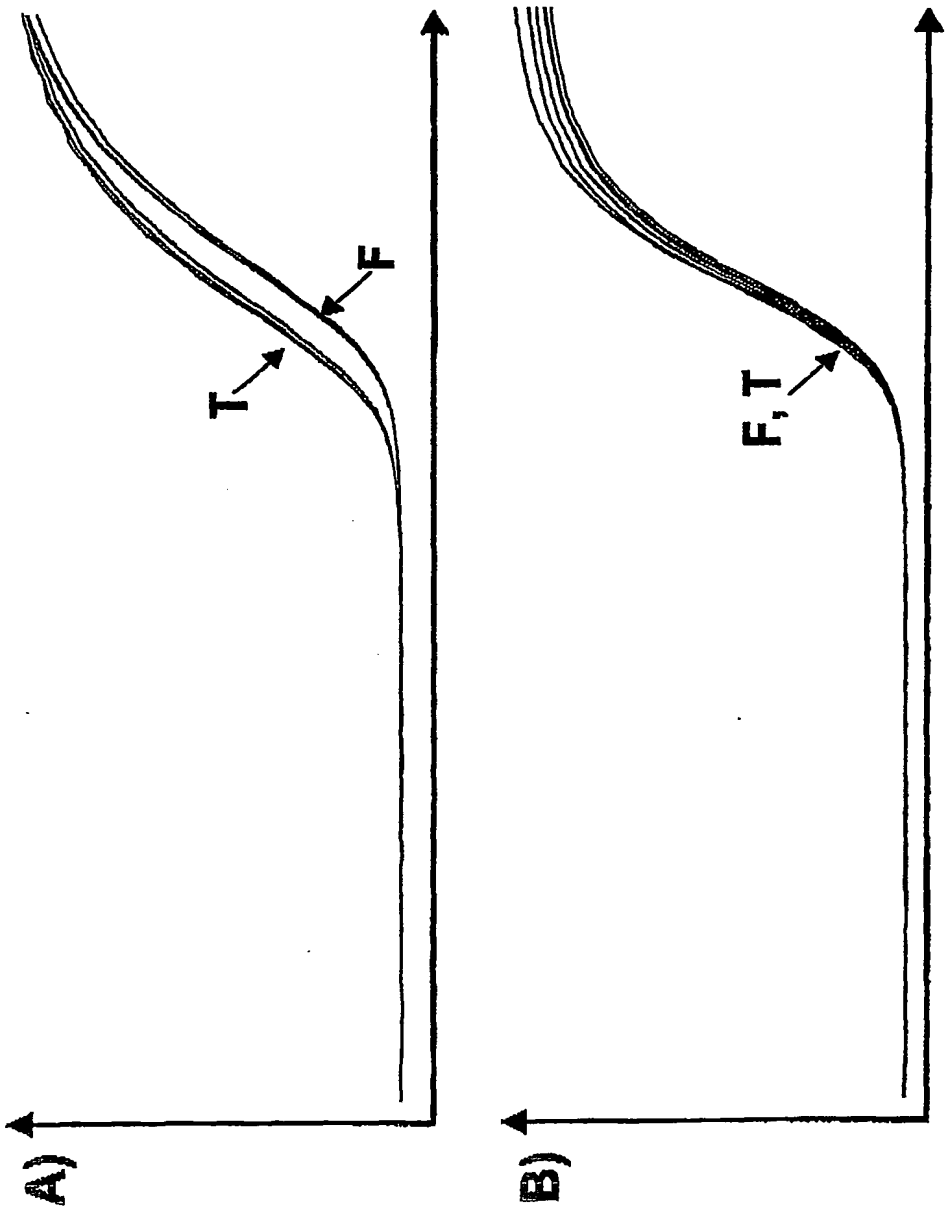

Figure 3: Verification of differential expression of the minor vault protein ADPRTL1 gene by quantitative PCR

Figure 4: Nucleotide sequence of SEQ ID NO: 1

Length: 35 bp

1 AATCTAGGAA TATTCCCTGG GCTTTTGAGG CAATC

Figure 5: Schematic alignment of SEQ ID NO: 1 with minor vault protein ADPRTL1 cDNA (GenBank accession number AF057160)

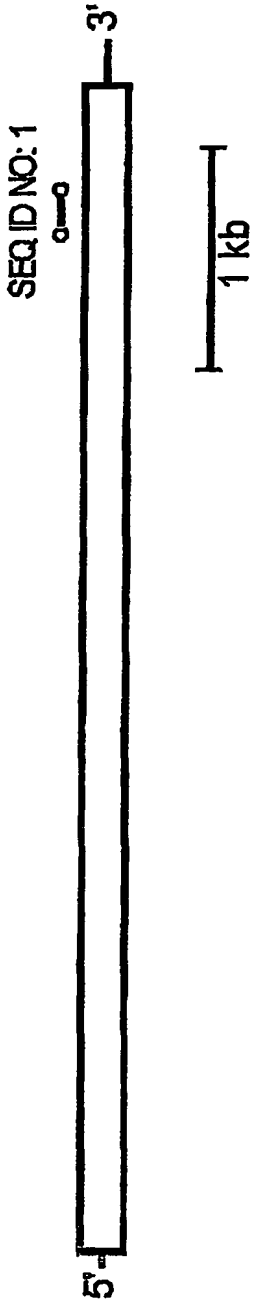

Figure 6: Alignment of SEQ ID NO:1 with minor vault protein ADPRTL1 cDNA (AF057160)

```
   3 TCTAGGAATATTCCCTGGGCTTTTGAGGCAAT 34
     || |||||||||||||||||||||||||||||
5027 TCCAGGAATATTCCCTGGGCTTTTGAGGCAAT 5058
```

Table 1:

sample	Δ–fold (temporal / frontal cortex)
control 1	0.77
control 2	0.98
control 3	0.93
control 4	1.02
control 5	1.23
patient 1	0.91
patient 2	1.62
patient 3	1.69
patient 4	1.33
patient 5	0.91
patient 6	1.53
patient 7	1.55